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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Kirsch, et al.
Appl. No.	:	09/924,396
Filed	:	August 6, 2001
For	:	IRON REGULATING PROTEIN-2 (IRP-2) AS A DIAGNOSTIC FOR NEURODEGENERATIVE DISEASE
Examiner	:	Chernyshev, Olga N

Group Art Unit 6247

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: United States Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202, on

February 20, 2004
(Date)

Jennifer A. Haynes, Registration No.: 48,868

DECLARATION UNDER 35 C.F.R. §1.132

United States Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

1. This Declaration is being submitted to demonstrate that there is significant evidence showing that the claimed invention can be used for both Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI) and that it will work to diagnose and identify a predisposition for such diseases by using a sample containing peripheral blood cells. Previously only one experiment was provided to the Examiner to support this method. However, in addition to that evidence (included as Exhibit B), we now have evidence from two other independent experiments which used material from patients whose diagnosis was performed using the enclosed clinical dementia rating. These are included as Exhibits E and F.

2. My name is Wolff M. Kirsch, M.D., and I am an inventor on the above-identified patent application and am familiar with the prosecution history .

3. I have extensive experience in the field of neurobiology as evidenced by my curriculum vitae attached as Exhibit A to my previous declaration.

4. Prior to the present invention, both AD and MCI have been notoriously difficult to diagnose. They typically require hours of extensive cognitive, psychometric, and physical tests which serve to identify the presence of AD and/or MCI, and rule out the possibility of other diagnoses. These tests are the most reliable in later stages of the diseases when phenotypic/behavioral changes can be seen. Thus, the invention, which provides a non-invasive method of diagnosis using molecular signs of disease is needed to reduce the time and expense as well as the patient discomfort associated with the psychometric tests. Compared to hours of analysis by a Neurobiologist, a simple lab test on a patient sample, would be a clear improvement to the cost, the patient comfort, and the Doctor's time.

5. The enclosed immunocytochemistry micrograph (presented to the Examiner in a previous Declaration) (exhibit B) provides strong evidence that peripheral blood cells can be used to identify the abnormal levels of IRP-2 produced by AD patients. The micrograph shows that lymphocytes stained with IRP-2 antibodies produce a clearly different pattern for Alzheimer's patients as compared to controls. This data was obtained following the enclosed protocol (exhibit C). The patient used in the study was diagnosed as shown in the attached exhibit D. Exhibit D contains 11 pages of tests which were performed on the Alzheimer's patient in 1992-1993 to identify the symptoms of Alzheimers and to rule out any other possible causes of such symptoms. The tests included liver tests, blood tests (SGOT is a metabolic test), MRI, EEG, and CT of brain to rule out brain tumor and stroke, and a physical assessment (see page 7 of the geriatric assessment) to rule out other causes. The geriatric assessment concluded that the patient had mild cognitive impairment. Subsequent tests have confirmed the diagnosis and the disease has now progressed to full AD. The patient is now living in a home and requires constant supervision due to the progression of the disease.

6. A second test was done comparing normal and AD peripheral blood lymphocytes by flow cytometry. The results are shown in Exhibits E and F. Exhibit E represents expression of Transferrin receptors on one axis and IRP-2 on the other axis in lymphocytes from normal control samples as measured by flow. Exhibit F represents expression of Transferrin receptors on one axis and IRP-2 on the other axis in lymphocytes from an AD patient as measured by flow cytometry. cytometry. For purposes of this application, the axes relating to transferrin receptor are not relevant. Exhibit E shows that in a normal control the expression of IRP-2 was consistently above the line drawn at about 2×10^2 (arbitrary units. Exhibit F shows

that in an MCI patient, there is an increase in expression of IRP-2 of at least two orders of magnitude. The patient used for this test was diagnosed as having MCI/borderline AD using the Clinical Dementia Rating (CDR) scale which is provided as Exhibit G. This patient was identified as having a CDR of 1.0.

7. The Clinical Dementia Rating scale is shown herein as Exhibit G. Pages 1-9 allow the clinician to more quantitatively identify how the patient rates on the Table on page 10 in the six categories. The scale for scoring the patient is from 0 to 3.0, with 0 being in the normal range and 3.0 being someone with severe Alzheimers disease. A CDR score of 0.5 is defined as having MCI. Ratings of 1 and 2 can identify cases which have mild or moderate Alzheimers.

8. A third test looked at the relative fluorescence given by PE labeled anti-IRP-2 antibody (IDD) was identified in a number of control and two MCI-AD patients which had CDI scores of greater than 2.5, severe Alzheimers. Peripheral blood lymphocytes from these patients and the controls were treated with IRP-2 antibodies and Transferrin receptor antibodies and the expression was analyzed by FACS analysis. The results are presented in Table 1.

Table 1

	Control	*MCI-AD*
N	23	2
Mean ± SD	1.9 ± 1.2	20.3
		7.6

Because of the method used for the analysis of expression of IRP-2, the values shown in the control samples are actually equivalent to nonspecific binding. Thus, in control lymphocytes, which are not metabolically active (not actively dividing), there is little to no expression of IRP-2. However, it is clear from Table 1 that the AD patients lymphocytes showed a significant increase in the expression of IRP-2, up to 10-fold in one patient, although even the four-fold difference seen in the other patient is significant. Thus, the results shown in Table 1 confirm that IRP-2 is over-expressed in the peripheral blood lymphocytes of MCI/AD patients.

6. It is clear from the above three experiments that diagnosis of AD can be made by identifying the levels of IRP-2 in the peripheral blood lymphocytes. The levels were identified in

three different manners on four different AD/MCI patients using patient samples containing peripheral blood cells at all stages of the disease, even at a very early stage in the disease.

7. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 Title 18 of the United States code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 2/17/04

Wolff M. Kirsch, M.D.

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